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Title: The invasive, non-native slipper limpet *Crepidula fornicata* is poorly adapted to sediment burial

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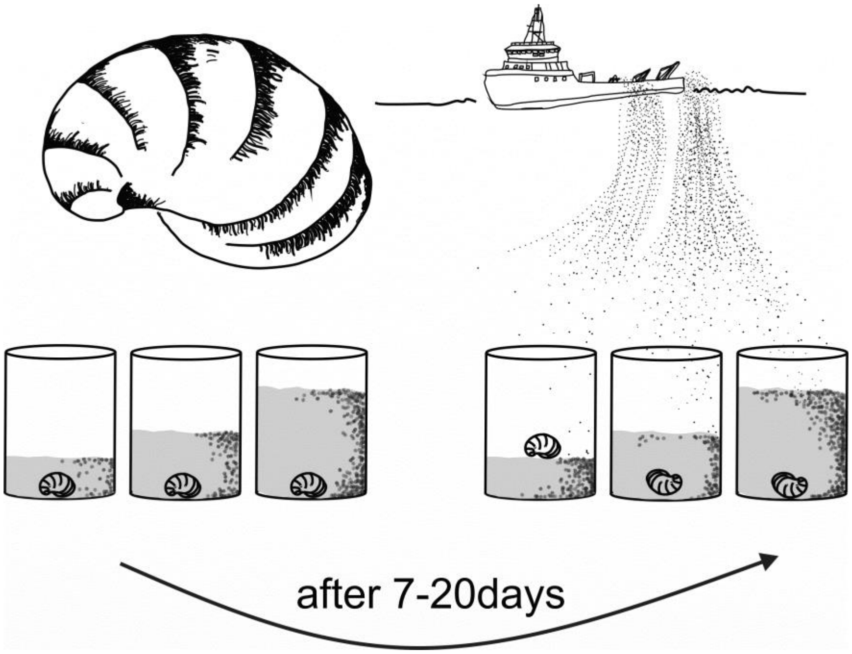
GRAPHICAL SUMMARY



ABSTRACT

The American slipper limpet *Crepidula fornicata* is an invasive, non-native species (INNS) abundant along the European coast. Its further distribution may be facilitated by activities such as dredging and spoil disposal, and the aim of this study was to assess whether *C. fornicata* is able to survive sediment burial. The slipper limpet was found attached to hard substratum in intertidal areas, but it was absent at a nearby subtidal dredge spoil site. In laboratory experiments 22% of *C. fornicata* emerged when buried under a 2cm sediment-layer; only half of them survived. When buried under ≥ 6cm none re-surfaced or survived. The results provided evidence that *C. fornicata* is poorly adapted to adjust its vertical position in sediment and is killed by sudden burial underneath 2 to 6cm of sediment. The combined laboratory experiments and field surveys suggested that *C. fornicata* has limited scope to survive the dredge spoil disposal process.

KEY WORDS

Crepidula fornicata, Swansea Bay Tidal Lagoon, dredge spoil disposal, coastal infrastructure, invasive non-native species

1. INTRODUCTION

1.1 Invasive non-native species

Non-native species (NNS) are not naturally found within a certain area and are also referred to as ‘non-indigenous’, ‘alien’ and ‘exotic’ species (Manchester & Bullock 2000). An invasive NNS (INNS) is a species that passed all stages of the invasion process including its release into a new environment, establishment and subsequent spread (Richardson et al. 2000, Bohn et al. 2015). INNS can cause harm to the environment and are regarded as one of the biggest threats to global biodiversity by outcompeting and dominating native species and often entire ecosystems (Thouzeau et al. 2000, Bax et al. 2003). Globalisation and human activity have both accidentally and deliberately transported INNS across major geographic barriers for centuries (Decottignies et al. 2007, Mineur et al. 2012). It is estimated that at any one time, 10,000 species are in transit around the world in ballast water, making it almost impossible to control the spread of species to new habitats (Manchester & Bullock 2000, Bax et al. 2003). More than 90 marine and brackish NNS have been identified in Britain and Ireland alone (Cook et al. 2015). Many NNS bring diseases, modify habitats and affect ecosystem functioning and can have indirect interactions with intermediate and top predators (Cook et al. 2015, Grason & Buhle 2016). The extent to which a NNS impacts a community depends on its interactions with native species (Grason & Buhle 2016).

The American slipper limpet, *Crepidula fornicata* is one of the most invasive non-native sessile invertebrates in Europe (Dupont et al. 2007). It is a suspension-feeding marine gastropod native to North America (Hancock 1969, Clark 2008). Its shell grows up to 50mm in length, 25mm in height, with a kidney shaped aperture and individuals attach to each other forming stacks (Clark

2008) (Figure 1). Human-mediated transport and its long-lived, free-swimming planktonic larvae have caused it to spread rapidly throughout Europe (Untersee & Pechenik 2007, Rigal et al. 2010). In the UK, *C. fornicata* extends from Pembrokeshire to Yorkshire including the Bristol Channel (Clark 2008). Hotspots include the Solent and Essex where *C. fornicata* forms a carpet over the seafloor, producing cohesive pseudofaeces as it filter feeds (Hancock 1969, Thouzeau et al. 2000, Clark 2008, Syvret & FitzGerald 2008). In the UK *C. fornicata* was introduced to Essex attached to oysters, *Crassostrea virginica*, between 1887 and 1890 and is now well known as their most abundant competitor (Orton 1912, Clark 2008, Bohn et al. 2013). The limpet can be found in most oyster producing areas in England and Wales where it occurs in enormous numbers (Hancock 1969, Thieltges 2005, Clark 2008). The limpet competes with oysters and other suspension feeders for space and food (Hancock 1969, de Montaudoüin et al. 2001, Moulin et al. 2007). Populations of the blue mussel *Mytilus edulis* can decrease dramatically when overgrown by slipper limpets (Nehls et al. 2006). The influence of *C. fornicata* on commercially important shellfish species can have huge economic implications (Thieltges 2005). *C. fornicata* modifies the nature and structure of habitat through biodeposition and the accumulation of its shells, often creating an unsuitable substratum for many native species (Thieltges 2005, Valdizan et al. 2009).

Its success can be explained by its strong reproductive viability and opportunistic feeding strategies together with the fact that it has few natural predators (Dupont et al. 2007, Clark 2008, Syvret & FitzGerald 2008, Valdizan et al. 2009). It is also tolerant to a wide range of salinities (Syvret & FitzGerald 2008, Rigal et al. 2010) and is found attached to a variety of substrates in the low intertidal and subtidal (Bohn et al. 2013, Cook et al. 2015). *C. fornicata* is a protandrous hermaphrodite that breeds from February to October and has a long-distance dispersal ability (Dupont et al. 2007). The availability of suitable substratum for settlement is crucial in determining its distribution (Barnes et al. 1973).

1.2 Methods of controlling the spread of *Crepidula fornicata*

Numerous methods have been employed to eradicate *C. fornicata*. Earliest attempts focused on eradication by dumping dredged *C. fornicata* above the high water mark and removing them by hand (Hancock 1969, Bolam et al. 2010, Cook et al. 2015). Since the 1950s, brine dipping has been trialed (Syvret & FitzGerald 2008); brine immersion for over 5 minutes resulted in 100% mortality (Syvret & FitzGerald 2008). This method is however not practical, especially for large amounts of material (Cook et al. 2015). Other attempts crushed *C. fornicata* stacks and fed their flesh to scavenging birds, or it was used as whelk bait (Hancock 1969, Clarke 2008, Valdizan et al. 2009). Chain riddles were used to break up stacks in Kent and Essex (Cook et al. 2015). This disturbance had, however, the unintended consequence to act as a dispersal vector for *C. fornicata*, further exacerbating the problem (Clark 2008, Cook et al. 2015). The slipper limpet was successfully eradicated from a commercial mussel lay in Wales, UK, by smothering with seed mussels of double the usual stocking density (Syvret & FitzGerald 2008, Cook et al. 2015). In the United States, INNS including *C. fornicata*, have been smothered with heavy duty polythene sheeting and then relayed with oysters (Hancock 1969), but this method was extremely costly and time consuming.

1.3 Dredge spoil disposal

The disposal of dredged material during the construction and maintenance of coastal infrastructure represents a significant problem in coastal management (Marmin et al. 2014, Callaway 2016). More than 40 million tons of sediment must be disposed of appropriately each year (Bolam 2011). Following dredge spoil dumping, changes in benthic communities are commonly reported since many species are smothered with sediment (Hutchinson et al. 2016). The greatest ability to emerge from burial for a range of macroinvertebrates is 2cm depth (Hendrick et al. 2016). Changes in the community structure are not restricted to the site of disposal and are often found kilometers away from the dumping area (Hendrick et al. 2016). The ability of species to escape burial through vertical migration is not well understood (Bolam 2011). The tolerance and responses of species to burial are species specific and cannot be generalized; species tolerance to burial depends on its adaptation and behaviour (Hendrick et al. 2016). Following burial, benthic invertebrates may recover by vertical or lateral migration and/ or the planktonic recruitment of larvae (Bolam 2011). Emergence from sediment burial is central to the chance of survival since failure to re-surface is assumed to eventually lead to death (Bolam 2011, Hendrick et al. 2016).

During the construction and maintenance of coastal infrastructure dredged spoil is disposed at designated sites. Dredged material may contain INNS, but legislation prohibits their release and spread (http://www.legislation.gov.uk/ukpga/1981/69/section/14). However, *C. fornicata* may not survive the dredging and disposal process. We hypothesized that smothering methods may kill any alive *C. fornicata* in dredge spoils. Whilst some speculative assessment of the intolerance of *C. fornicata* to burial has been made there is a lack of evidence to support assumptions for informed management decisions (Johnson 1972, Rayment 2008, Cook et al. 2015, Syvret & FitzGerald 2008). The aim of this study was therefore to assess the mortality of *C. fornicata* under sediment burial to determine whether smothering could be an effective way to prevent its spread. A multifactorial experiment was conducted to test burial intolerance using various burial depths and durations, and both stacks and individuals of *C. fornicata* were assessed.

This study had the following objectives

i) Identification of the preferred habitat of *C. fornicata*;
ii) Assessment of *C. fornicata* presence at a dredge spoil disposal site;
iii) Quantification of survival rates of *C. fornicata* under different sediment burial regimes.

2. MATERIALS AND METHODS

2.1 Study site

Intertidal and subtidal *C. fornicata* surveys were carried out in Swansea Bay, South Wales, UK (Figure 2). Swansea Bay is located along the northern coastline of the Bristol Channel and has the second largest tidal range in the world with mean spring tides of 8.5m and neap tides of 4.1m (Collins et al. 1979, Smith & Shackley 2006). The bay stretches roughly 12km from Mumbles Head to Port Talbot with the Eastern side facing directly towards the Atlantic Ocean (Collins & Banner 1980, Cefas 2011). A complex hydrodynamic system arises from the bathymetry and configuration of the Bay (Collins et al. 1979). A rectilinear semi-diurnal tidal system reverses the offshore flow resulting in an anticlockwise gyre within the western part of the bay and an area of divergence on the eastern side (Smith & Shackley 2006). The embayment is shallow with depths rarely exceeding 20m and the currents are strong with limited exchange of water between the Bristol Channel and open sea (Ferentinos 1978, Collins & Banner 1980, Lindsay et al. 1980). Inner Swansea Bay consists primarily of fine and medium sand with some mud (Smith & Shackley 2006). A dredge disposal site or spoils ground is situated in outer Swansea Bay approximately 13km from Swansea and covers an area of 6 hectares (Figure 2). It is mostly used to discard materials from maintenance dredging of shipping lanes and consists of primarily fine sand and mud.

2.2 *Crepidula fornicata* habitat preference survey

Between March and April 2016 the intertidal area of 5 sites along Swansea Bay were quantitatively surveyed for the presence or absence of *C. fornicata*. Sites were chosen to cover a variety of habitat types. However, the survey focused on intertidal areas characterised by rocky boulders, shell debris and glacial till since the slipper limpet is known to require attachment surfaces (Clark 2008, Bohn 2012, Bohn et al. 2015). At each of the 5 sites, down-shore transects were located at 100m intervals, and each transect measured between 400m and 800m in length depending on the expanse of the intertidal area. Along each transect, stations were plotted at 50m intervals apart. At each station, 3 x 0.25m^2 quadrats were placed randomly and surveyed. Where present, the number of *C. fornicata*, the nature of the attachment substrate and the size of individuals was recorded. All *C. fornicata* individuals and stacks found within each quadrat were counted. The number of juveniles and adults was also recorded; juveniles were defined as individuals <1cm in their largest linear dimension, adults were >1cm. The size of 1cm was an arbitrary number based on an easily distinguishable size and the fact that newly settled *C.*

fornicata measure 1-5mm (Pechenik & Heyman 1987). A total of 27 transects were surveyed between Mumbles, Swansea West, West Cross, Black Pill and the *Sabellaria alveolata* reef at Swansea East (Figures 2,3). This amounted to a total of 770 x 0.25m^2 quadrats being surveyed at 262 stations.

2.3 Dredging of spoil ground

The Swansea Bay outer spoils ground is used to discard dredged material which could potentially contain individuals of the invasive slipper limpet. The spoil ground was surveyed for *C. fornicata* on the 12th July 2016 (Figure 3). There are no known records of *C. fornicata* at the spoils ground to date, and hence the survey covered as much area as possible in an attempt to detect any signs of the non-native being present.

Samples were obtained using a 75cm oyster dredge with 4cm metal mesh, 2cm teeth and an opening mouth of 27cm. Station locations and the direction of each tow was determined randomly and depending on the conditions of the wind and tide and the timeframe available to cover as much of the spoils ground as possible. The duration of each tow was initially standardised to 5 minutes at the bottom. However, very little material was picked up in the first 4 tows and therefore duration was increased to 10 minutes for tows 5-12. An additional 5-minute control dredge sample was taken closer inshore at Mumbles, known for the presence of *C. fornicata*. This was to ensure that the oyster dredge would retrieve *C. fornicata* where present. All material picked up in the dredge bag was closely examined for *C. fornicata* and trawl fullness was recorded as a percentage. Associated epifauna was recorded and a photo of each dredge bag was taken. A total of 100 minutes of towing at a towing speed of two knots amounted to a total distance of 6,173 metres being surveyed for *C. fornicata* at the spoils ground.

2.4 Experimental burial of *C. fornicata*

Laboratory experiments manipulated burial depth and duration to assess mortality under sediment burial of *C. fornicata*. Three burial depths were tested: shallow (2cm, n=27), medium (6cm, n=27) and deep (12cm, n=27). Each depth was tested over three durations of 2 days (n=27), 7 days(n=27) and 20 days (n=27) in separate tests for each depth and duration (n=5, Figure 5). Burial depths were chosen based on the expected potential vertical migration of *C. fornicata*, which was estimated to resemble similar species' ability to escape burial (Nichols et al. 1978, Chandrasekara & Frid 1998, Bolam, Schratzberger & Whomersley 2003).

Specimens for the experiment were collected as stacks of *C. fornicata* from the intertidal area of western Swansea Bay (51°34'48.13" N, 3°59'21.95" W). All individuals were acclimatised in seawater for 1-2 weeks in the Swansea University aquarium laboratory. Water temperature was approximately 18 °C throughout the experiments. Stacks were chosen at random from the acclimatisation tanks and allocated to a pre-determined burial treatment. Experiments were separately carried out on single individual (experiment 1, n=5) and on stacks of *C. fornicata* (experiment 2, n=4).

Experiment 1 on single *C. fornicata* involved removing all but the bottom individual attached to the substrate using a blunt diving knife. *C. fornicata* were not removed from their attachment substrate before burial. They were measured along their largest linear dimension to 1 mm resolution using Vernier callipers. Stack height was measured in experiment 2 and the number and size (adult/ juvenile) of individuals within each stack noted. Substrate of attachment was also recorded for all individuals and stacks along with the timings of the experiment. Experiments were carried out in the aquarium research laboratory at Swansea University from June to August 2016. *C. fornicata* were placed into individual tanks in water depth of 50cm. A flow-through system and airstones prevented water stagnation. All combinations of burial depth and burial duration were replicated 5 times in experiment 1 and 4 times in experiment 2.

Sediment was collected by hand from the top 5cm of sediment in the intertidal of western Swansea Bay (51°34’49.39” N, 3°59’57.89” W). Local sediments were collected since Bolam (2011) showed that depositing non-native sediments impaired survival severely. Mixed sediment directly from Swansea beach was used for both experiments to replicate the local conditions as closely as possible. Sediments were defaunated by oven-drying at 65 degrees C° for 5 days and then cooled. Sediment was placed at the bottom of each tub as a base layer. *C. fornicata* were manually buried according to a predefined burial treatment. Burial depth was measured from the highest point of the individual in experiment 1 and the highest point of the stack in experiment 2. All trials were run alongside controls with un-buried individuals. At the end of each burial treatment, any emergences were recorded and individuals were carefully removed. Survival was assessed following a method developed by Syvret & FitzGerald (2008), which records *C. fornicata* as dead when individuals can not adhere to the basal connection. In most cases this was clear because the *C. fornicata* cleanly separated from their attachment substratum, but in some cases dead individuals remained suctioned to their base. In these cases, gentle finger pressure was used, and if they could not be separated from their substratum they were recorded as still living

(Syvret & FitzGerald 2008). The survival of each individual within the stack was recorded for experiment 2 along with its age (juvenile/ adult). In experiment 2, the stack was recorded as still living as long as at least one of the individuals within the stack survived.

2.5 Data Analysis

The abundance of *C. fornicata* was mapped with ArcMap version 10.3 (ESRI, California, USA) and positions with and without *C. fornicata* were superimposed on a Phase I GIS layer provided by Natural Resources Wales (NRW, UK) to show the biotopes associated with *C. fornicata* presence. The tow path of each haul at the dredge spoil site was mapped onto a habitat map provided by NRW to allow for spatial comparisons between dredge path and the substrate within the spoils ground. Dredge tow paths were then mapped onto Admiralty chart 1161 for Swansea Bay to show the area covered within the outer spoils ground.

Data were analysed to study the effects of burial depth and burial duration on the mortality of *C. fornicata* under sudden burial. As a control for unknown factors causing mortality in the laboratory environment, mortality levels of non-buried limpets were monitored. As all control specimen survived (n=27, 0% mortality) the control data was excluded from further analysis.

For all subsequent analysis, a binomial generalized linear model (using the GLM function in R version 3.3.1) with a logit link function was used. Two separate analyses were run to test a) the mortality of individual limpets (experiment 1) and b) stacks of *C. fornicata* (experiment 2). The following script was run in R:

glm (formula = Mortality ~ Depth + Duration, family = binomial)

The same binomial GLM was used to test whether the level of mortality of *C. fornicata* individuals was significantly different to mortality levels for stacks. In all models the one with the lowest Akaike Information Criterion (AIC) was considered to be the model which described the experimental data best. The probability of emergence from burial was also tested against burial depth and duration using a binomial GLM with logit link function. Responses of limpets in each treatment were analysed by fitting models with all terms, both with and without interactions among variables.

3. RESULTS

3.1 Intertidal Surveys

A total of 1416 *C. fornicata* individuals were recorded during the intertidal surveys. The slipper limpet was present at 30.2% of stations surveyed (n = 262) and 18.2% of quadrats (n = 770) from all 5 survey sites in densities up to 412 individuals per m^2 (Fig. 5). No *C. fornicata* were recorded at sandy site Blackpill. *C. fornicata* density was highest at the Swansea East site, especially towards the breakwater, and it was generally more abundant towards the lower shore. According to the Phase I data map, the majority of *C. fornicata* were recorded along mussel beds, muddy sandy shore, fucoids and biogenic reefs. However, in this survey few mussels were recorded in the intertidal area, which contradicts the Phase I habitat map from 2001-2004 surveys (Swansea West) and 2003 (Swansea East). The area labelled as mussel beds in the phase 1 data was, however, coarse material and provided settlement substratum for *C. fornicata*. The slipper limpets were attached to stones and empty mollusc shells of *C. fornicata*, *Mya arenaria*, *Pecten maximus*, *Litorina littorea*, *Mytilus edulis* and other bivalves. The majority were attached to stones (64%) followed by empty *C. fornicata* shells (26%). Overall, 39.7% of *C. fornicata* recorded were juveniles (<1cm; n = 562) and 60.3% were adults ($\geq$1cm, n = 854).

3.2 Subtidal survey at dredge spoil site

A total of 4,582m^2 of the spoils ground was dredged in a cumulative 6.1 km tow in an attempt to find out whether *C. fornicata* was present within the area (Figures 1 & 3). The dredge fullness was always less than 10% at the spoils ground; some dead shells and cobbles were picked up. No benthic fauna was recorded in 6 of 12 dredge tows. Individual specimens of the following epibenthic species were present in the remaining tows: *Asterias rubens, Ophiothrix fragilis, Aphrodita aculeate* and *Pagurus bernhardus*. However, no *C. fornicata* were found at the spoils ground; one empty, broken *C. fornicata* shell was picked up. The control dredge tow at Mumbles covered 309 metres and picked up 97 *C. fornicata* individuals (78 adults and 19 juveniles). There were 25 *C. fornicata* stacks in total in the control dredge. Other recorded species in the control dredge were *Pagurus bernhardus*, *Styela clava*, *Porcellana platycheles, Cancer pagurus, Asteria rubens, hydroids*, *pycnogonids* and *barnacles*. Dredge fullness was 75% following the tow at Mumbles.

3.3 Laboratory Experiments

Emergence from Burial

Burial depth had a significant effect on the emergence of *C. fornicata*, that is, when *C. fornicata* escaped from burial by moving to the surface of the sediment (GML: z = 2.662, P = 0.008). 22%

of *C. fornicata* (four individuals, three stacks) emerged from 2cm sediment coverage, but none from 6 or 12cm burial. Of the emerging *C. fornicata* which had been buried under 2cm sediment 7% emerged after 7 days and the remaining 15% after 20 days. However, of the 7 individuals and stacks only four were alive when analysed (one individual and three stacks). The number of individuals in each stack did not have a significant effect on the ability of *C. fornicata* to emerge from burial (GLM: z = 0.862, P = 0.389, n = 36).

Mortality of C. fornicata due to sediment burial

No *C. fornicata* died in non-buried controls (n=27) while a total of 81.5% of *C. fornicata* (n = 81) died in burial treatments (proportion test: P = 3.021e-13). The probability of mortality in *C. fornicata* under burial significantly increased with increasing thickness of the sediment layer (GLM: z = 2.167, P = 0.03, n=27 per depth) (Fig. 6). Three individuals were alive after 2 days under 12cm sediment burial but none had survived after 7 or 20 days. However, generally duration of burial did not have a statistically significant effect on the mortality of *C. fornicata* (GLM: z = 1.894, P = 0.058, n=27 per duration). No significant interaction was found between depth and duration on mortality (GLM: z = 0.506, P = 0.615).

Neither the size of buried individual slipper limpets nor the height of stacks had a significant influence on mortality (size of individuals GLM: z = -1.555, P = 0.12, n = 45; height of stacks GLM: z = 0.083, P = 0.934, n = 36). The size of individuals ranged from 2.8 – 4.5cm (n = 45) with an average size of 3.8cm. The average size of buried *C. fornicata* which survived the treatment was 4.0cm (n = 7) and for those that did not survive 3.7cm (n = 38) (Figure 7). Height of stacks was 1.2 – 6.6cm (n = 36) with an average height of 3.7cm. The number of individuals per stack varied from 2 – 15 individuals (n = 36) with an average of 6 ± 2.6, but the numbers of individuals in the *C. fornicata* stack again did not have a significant influence on the mortality of the stack (GLM: z = -0.866, P = 0.386, n = 36). Generally, there was no significant difference in the probability of mortality under sudden burial between individuals and stacks (GLM: z = -0.764, P = 0.444, individuals n=45, stack n= 36).

4. DISCUSSION

This study showed that the invasive, non-native slipper limpet *Crepidula fornicata* was present in intertidal habitats, but it was not found at a nearby subtidal dredge spoils disposal ground. Generally, benthic species can be severely impacted by dredge materials and traditional methods of discarding dredged spoils often result in burial depths that exceed the emergence ability of the resident fauna (Wilber et al. 2007). Disposal of sediment in thin layers less than 15cm deep potentially allows benthic species to laterally or vertically migrate through the sediment or to be passively transported to the surface (Chandrasekara & Frid 1998, Wilber et al. 2007).

4.1. Intertidal distribution of *Crepidula fornicata*

The slipper limpet *C. fornicata* was exclusively found in environments that offered hard substratum. The species showed habitat preferences for rocky grounds colonized by *Sabellaria alveolata* (honeycomb worm); over 80% of the recorded slipper limpets were present among this reef forming tube worm. *C. fornicata* and *Sabellaria* spp. are commonly recorded in parallel and appear to share habitat preferences (Schlund *et al.* 2016). There is so far no evidence of the nature of their relationship, whether they are, for example, competing for space or facilitating each other's presence. Highest densities of *C. fornicata* were found closest to a shelter-providing breakwater. This confirms *C. fornicata*'s preference for sheltered, shallow areas and its avoidance of high energy environments (Moulin et al. 2007, Rayment 2008, Clark 2008). *C. fornicata* is usually most abundant at the intertidal-subtidal interface (Rayment 2008, Blanchard 2009, Bohn 2012, Cook et al. 2015), and in this study, the majority of *C. fornicata* were also recorded at the mid and low shore. However, the species was found throughout the intertidal area, albeit in low numbers in upper intertidal regions.

C. fornicata require hard substrata for settlement and attachement (Bohn 2012, Bohn et al. 2012), which is critical in determining distribution (Barnes et al. 1973). Similar to previous studies, the majority of *C. fornicata* were found to be attached to stones (64%) and the empty shells of conspecifics (26%), with the remainder being attached to the shells of alive and dead bivalves and gastropods (Thieltges et al. 2004, Thieltges 2005, Moulin et al. 2007, Rayment 2008, Bohn et al. 2012).

4.2. *Crepidula fornicata* at sublittoral dredge spoils ground

A key motivation of this study was to establish whether *C. fornicata* was present at a site that is used to discard materials from maintenance dredging which could potentially contain slipper limpets. Generally, the dredge spoils site seemed to be an ecological desert with very little benthic fauna recorded in the combined 6.1km dredge tow covering 4,583m^2. No slipper limpets were found. In contrast, a single 309m control dredge tow at a site known to be inhabited by *C.*

fornicata contained 97 slipper limpets. While it cannot be rules out that individual *C. fornicata* may have been present in areas of the spoil disposal site not covered by this survey, it seems plausible to conclude that the site is not colonized by slipper limpets.

Our results support previous findings, where the benthic community was classified as “poor” or “bad” according to the Water Framework Directive classification at eleven locations in the outer Swansea Bay area near the dredge spoil disposal site (Callaway 2016); 90 other sites in the inner bay were classified at least “moderate” or “good”. It appears that disposing of spoils from maintenance dredging in the outer bay may negatively impact the benthic environment in its immediate vicinity. Dredging and the disposal of spoil tends to increase turbidity, changes the composition of sediment and mobilises heavy metals and other harmful materials depleting areas of biota (Marmin et al. 2014, Little et al. 2016). Deposited material often changes the characteristics of the seabed (Okada et al. 2009).

4.3. Smothering *Crepidula fornicata*

Laboratory experiments demonstrated that *C. fornicata* was to a limited degree capable of emerging from smothering with a 2cm deep sediment layer after a duration of 7-20 days, and it survived the temporal burial. In contrast, no *C. fornicata* buried under 6 or 12cm survived longer than 7 days. None of the tested individuals showed movements towards the sediment surface when buried under 6cm or 12cm of sediment, suggesting the level of sedimentation was too high for *C. fornicata* to reach the surface and escape from burial. The ability of *C. fornicata* to emerge from shallow (2cm deep) burial disagrees with past studies which stated that adult *C. fornicata* were unable to burrow or reposition themselves once covered with sediment (Cook *et al.* 2015). While *C. fornicata* is a sedentary, relatively non-mobile species, it is capable of movement. The slipper limpet shows two aggressive behavioural responses when threatened by the oyster drill gastropod *Urosalpinx cinerea*. It can lift its shell, extend its head and rasp an oyster drill with its radula (Pratt 1974). *C. fornicata* is also able to rotate constantly if mounted by an oyster drill and put pressure on the gastropod if it became trapped against an obstacle (Pratt 1974). These defensive maneuvers may explain the process by which *C. fornicata* was able to escape from 2cm sediment burial.

The ability of epifauna to re-surface is species specific and depends on motility, living position, tolerance of anoxic conditions and behavioural responses (Schratzberger et al. 2000, Hinchey et al. 2006, Bolam 2011). *C. fornicata*'s limited ability to emerge from smothering seems broadly in line with other epibenthic species. Bulk density and burial depth reach a critical threshold value above which animals cannot initiate an escape response, called "overburden stress" (Nichols *et al.* 1978). They seem generally unable to escape from burial of more than 1cm while infauna can escape from over 10cm (Chandrasekara & Frid 1998); the epibenthic gastropod *Hydrobia ulvae* is an exception being able to escape from 16cm of sediment burial (Bolam, Schratzberger & Whomersley 2003, Bolam 2011). On the other hand, the sessile bivalve *Modiolus modiolus* has no behavioural response to escape burial even from shallow depths although it is often found partially buried, while *Mytilus edulis* was able to escape from 2cm burial (Hendrick et al. 2016). It was suggested that the mussels were able to detect the depth of overlying sediment since they slowed down their vertical migration as they approached the surface of the sediment (Henrick *et al.* 2016).

There was no significant difference between the mortality under burial for stacks and individuals of *C. fornicata*. Further, the average size of buried individuals did not have a significant effect on mortality. This result differs from other epibenthic species such as mussels, where larger individuals are more capable of escaping from burial because they have fewer body lengths to travel (Hutchinson et al. 2016). Juvenile clams generally showed greater mortality under burial compared to adults as they had very limited ability to withstand smothering (Emerson et al. 1990). In contrast, adult venerid clams were less tolerant to burial compared with juveniles (Bellchambers and Richardson 1995). Generally, the number of juvenile and adult individuals in this study was limited and the question, whether or not there is a difference in their tolerance to burial ought to be revisited in further research.

Compromised feeding

C. fornicata show a variety of stress responses including reduced shell growth (Johnson 1972, Davies et al. 2009) and decreased metabolic rate (Davies et al. 2009), which suggests that they may be capable of adapting to burial treatments. It is possible that smothering compromises its ability to feed effectively. *C. fornicata* is primarily a suspension feeder which uses mucus threads to entangle particles on its gill filaments. These particles are then converted to food cords, grabbed by the radula and then consumed (Johnson 1972, Shumway et al. 2014, Cook et al. 2015). The feeding structures would become clogged under smothering of 5cm from the base of the stack (Rayment 2008). This could explain why no *C. fornicata* survived or emerged from burial under depths of 6-12cm. However, although energetically costly, *C. fornicata* are capable of clearing their feeding structures (Johnson 1972, Cook et al. 2015). The limpet is often extremely abundant in silty and muddy substrata and its deposition of pseudofaeces produces further silt, which seems to have no negative effect on the species (de Montaudouin et al. 1999, Thouzeau et al. 2000, Rayment 2008). Further, *C. fornicata* survived extremely turbid water conditions in the laboratory experiments where they kept their filtering structures clear of debris by excreting pseudofaeces (Johnson1972). Despite this ability, the slipper limpet is unlikely to feed effectively if completely smothered (Cook *et al.* 2015). In the current study, *C. fornicata*'s ability to feed may have been compromised, but still managed to escape and survive light burial.

Remaining buried and not attempting to escape from burial may not increase the chance of survival in the long term but it may save energy in the short term; energy could then be restored if natural water movements unburied individuals (Hutchinson et al. 2016). However, the energetic cost of starvation and migration may explain why 43% of *C. fornicata* in our study which had re-surfaced but were not alive when analysed.

Oxygen deprivation, temperature and sediment characteristics

It is plausible that *C. fornicata* under burial were experiencing hypoxic and/ or anoxic conditions. The presence of oxygen within the overburden sediment is likely to have huge consequence for the survival of species (Cottrell et al. 2016). The reaction and adaptation to anoxic conditions is however species specific and depends on states of activity (Theede 1973). Oxygen rapidly decreases while ammonia and hydrogen sulfide increase in deposited sediments (Bolam 2011). When unburied, *C. fornicata* were often surrounded by an anoxic black layer in this study, especially at deeper burial depths. Since 81.5% of *C. fornicata* under burial did not survive, it is likely that *C. fornicata* was intolerant to an anoxic and/ or hypoxic environment.

Resistance of invertebrates to hydrogen sulfide is significantly higher at lower temperatures and reduced pH (Theede 1973, Hutchinson et al. 2016). Higher temperatures mean an increase in metabolic demand which therefore leads to a higher mortality (Pfitzenmeyer & Drobeck 1967, Cottrell et al. 2016). Water temperature in the laboratory was 18 °C., and it is possible that the water temperature at the spoil ground is lower for much of the year. *C. fornicata* may be more tolerant to burial in the field. The time of year of spoil disposal could therefore have a significant effect on the survival of *C. fornicata* under burial. The timing of dumping can also influence how the sediment is dispersed (Lindsay et al. 1980, Rigal et al. 2010). Dumping sediment in attempt to smother *C. fornicata* may be less effective in winter when severe storms can suspend sediments, especially in embayments such as Swansea Bay which is shallow and muddy (Lindsay et al. 1980).

The organic content and grain size of the sediment also influences the tolerance of species to sediment burial (Turk & Risk 1981, Chandrasekara & Frid 1998, Bolam 2001, Cottrell et al. 2016, Hutchinson et al. 2016, Hendrick et al. 2016). Porous, coarse sediment has elevated oxygen flux rates which is likely to lead to an increased ability to vertically migrate (Cottrell et al. 2016). *Hydrobia ulvae*, for example, generally showed better vertical migration when the organic content of sediment was low (Bolam 2011). However, an increase in the sand content of dredged material had no noticeable effect on emergence in the studies by Bolam, Schratzberger & Whomersley (2003).

4.4 Further research

Survival of some species in the field has been reported as being different to their survival under laboratory conditions (Bolam 2011). The survival of the slipper limpet should therefore be further tested in field experiments. This would also allow testing for seasonal effects. The process of displacement, transport and dumping of *C. fornicata* from the dredge area to the spoils ground is likely to add to the stress and is likely to contribute to their vulnerability, including direct impacts such as the breaking-up of stacks and shell damage. Since spoil disposal sites are often deeper than dredged areas, effects of pressure change on *C. fornicata* need to be better understood. Further, more than 50% of the dead *C. fornicata* that were analysed following burial contained eggs. Further research is required as to whether these mature eggs would be able to survive if disposed off at sea, which would allow the spread of the species.

4.5 Conclusions & Recommendations

This study suggests that *C. fornicata* is fairly intolerant to sediment burial. Burial depth has a significant effect on both the re-surfacing and survival of *C. fornicata*. The probability of mortality significantly increased with increasing sediment overburden. No *C. fornicata* were found to be alive after 7 days under medium and deep burial, and individuals only emerged from 2cm sediment burial after 7 days or longer.

Given that *C. fornicata* did not survive burial deeper than 6cm, this study recommends smothering with a layer of material of at least this depth if the management objective specifies that no slipper limpets should stay alive. Since stacks of the gastropod were up to about 7cm high it would be prudent to increase the layer of deposits by that margin to make sure that the upper individuals are affectively covered. Still, the feasibility of this method must be viewed with caution. Current and wave action can uncover buried slipper limpets and it is debatable how accurately burial depth can be determined.

Generally, there is a trade-off between minimising negative effects of dredge spoil disposal on native benthic fauna and maximizing the amount of sediment deposited to ensure mortality of INNS such as *C. fornicata.*

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Figures

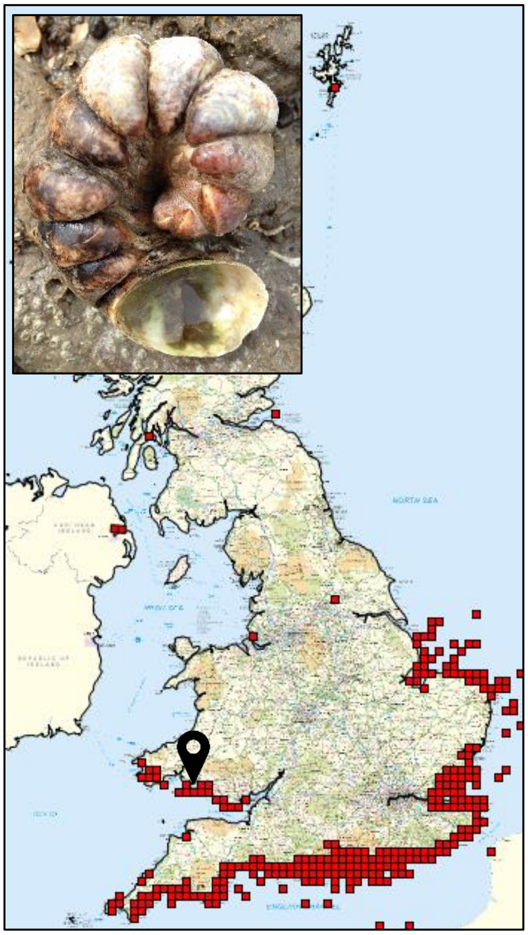


Figure 1. *Crepidula fornicata* stack (image) and records of the species' presence in the UK (map from National Biodiversity Network Gateway UK, 2011). Location icon marks study site Swansea Bay, Wales, UK.

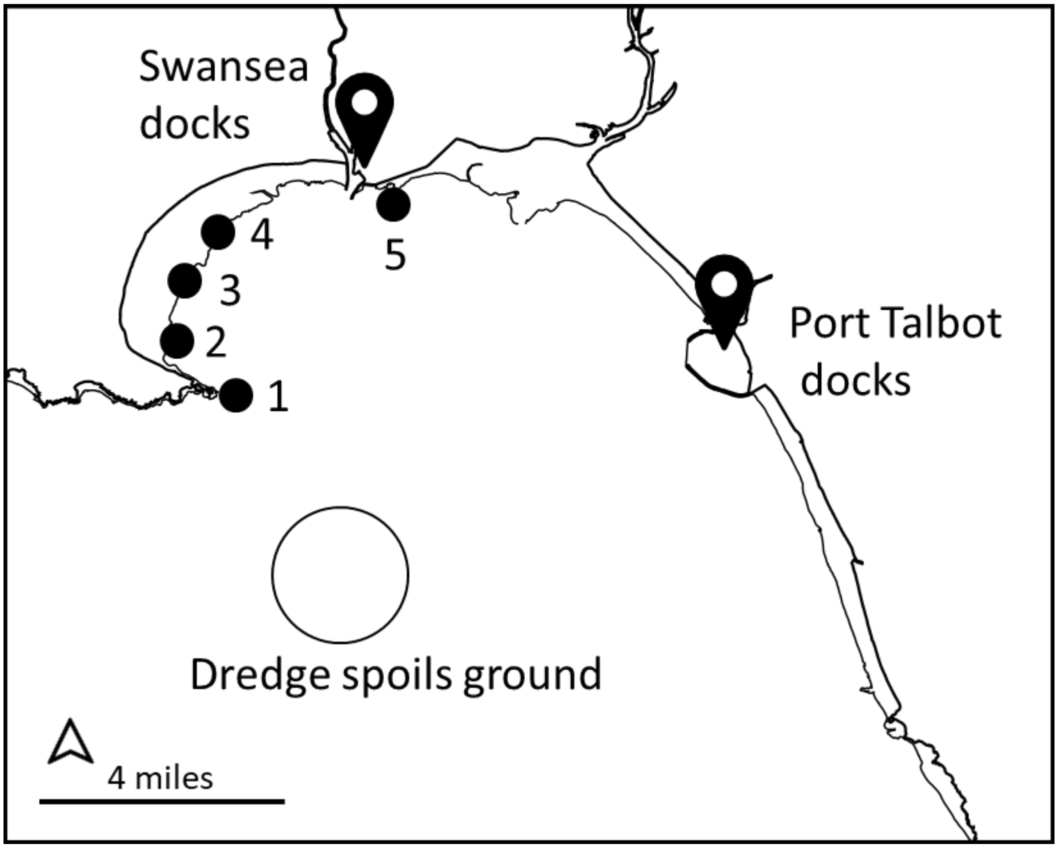
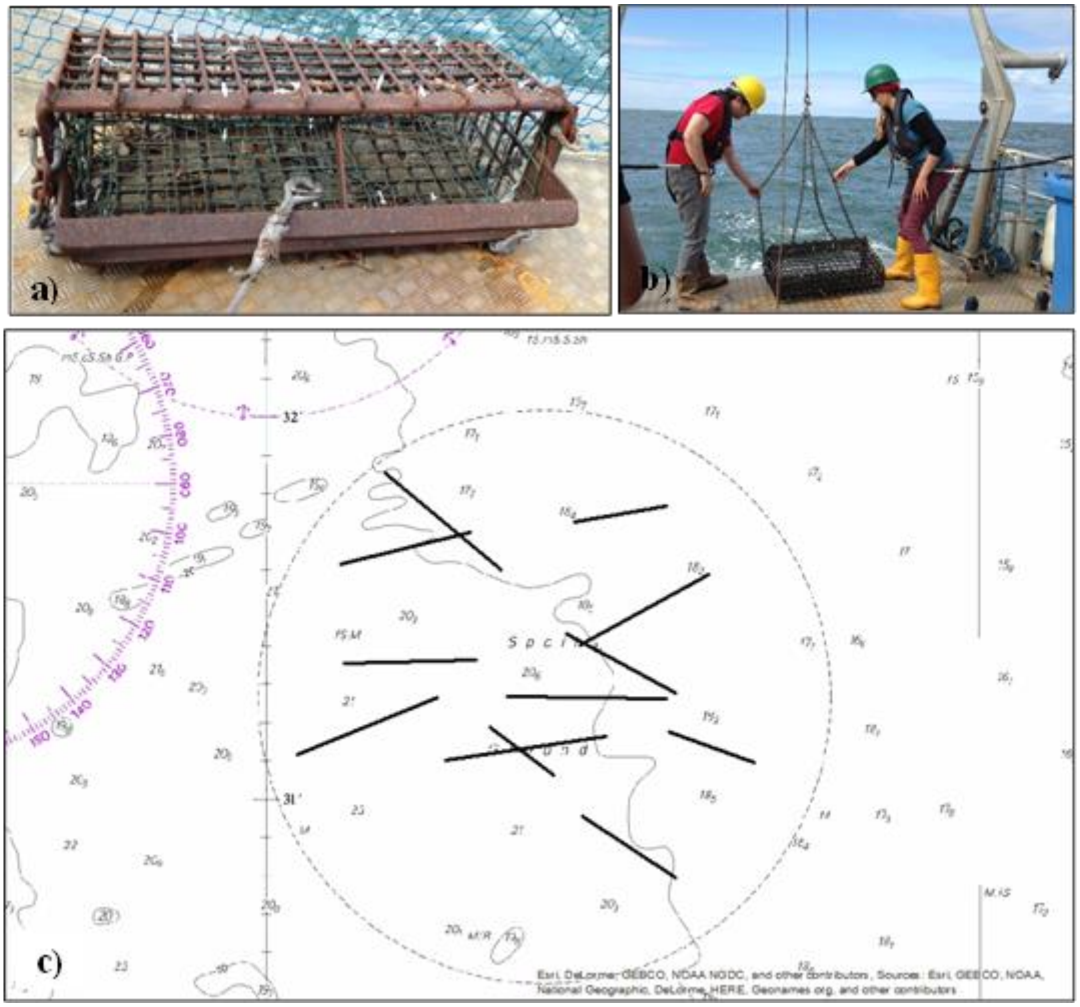


Figure 2. Study site Swansea Bay, South Wales, UK. Black dots indicate the location of the 5 intertidal sites surveyed (1. Mumbles, 2. Swansea West, 3. West Cross, 4. Black Pill, 5. Sabellaria East). The dredge spoils ground in the outer Swansea Bay is shown, where the subtidal surveys took place (see Figure 3).



Esri, DeLorme, GEBCO, NOAA NGDC, and other contributors, Sources: Esri, GEBCO, NOAA, National Geographic, DeLorme, HERE, Geonames.org, and other contributors

Figure 3. Dredge spoils site survey for *Crepidula fornicata*; a) Oyster dredge sampling equipment; b) deployment of oyster dredge; c) Swansea Bay outer dredge spoils ground from Admiralty chart 1161. The dashed circle outlines the spoils ground and black lines within the ground show the dredge tow paths.

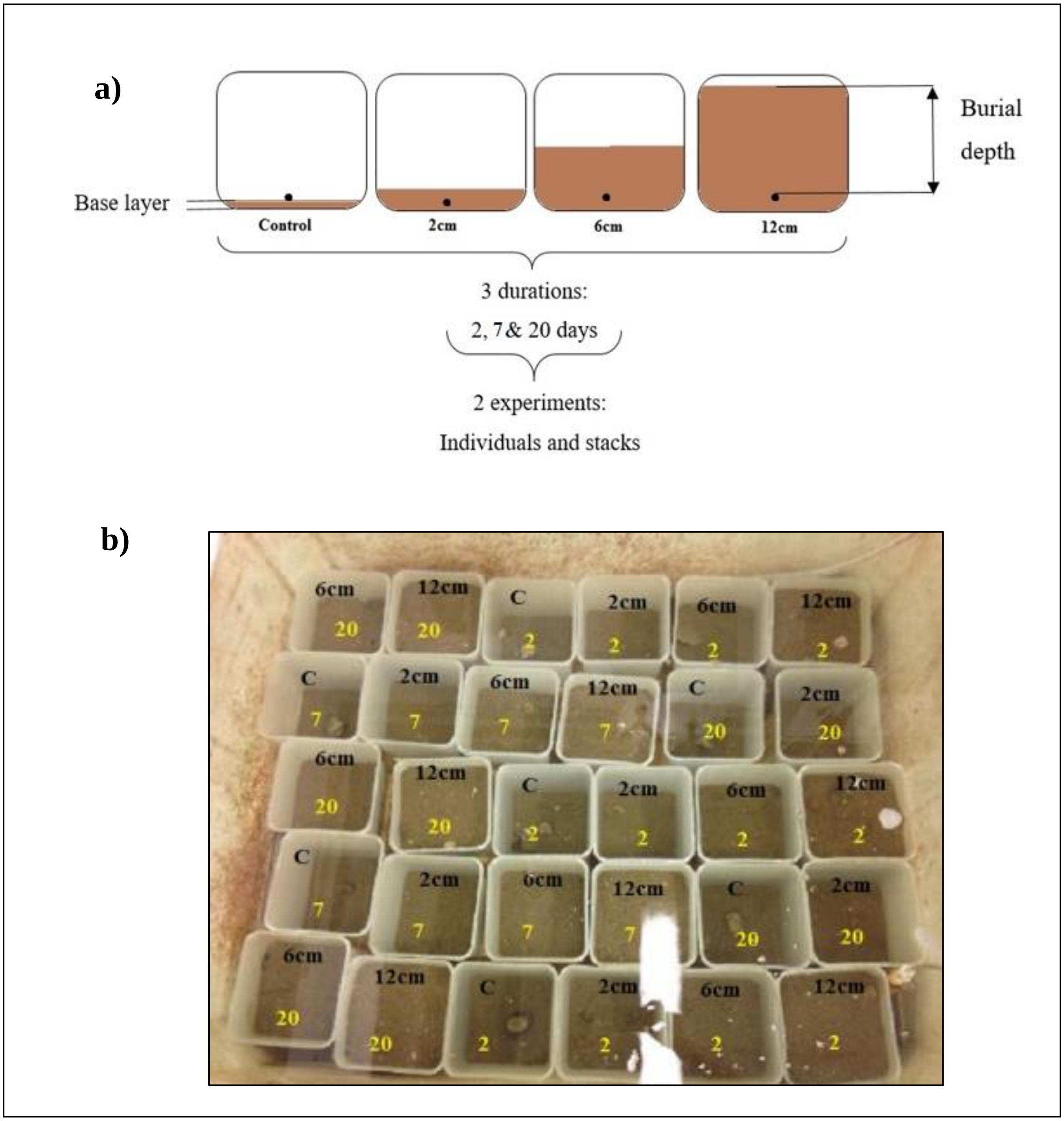


Figure 4. Laboratory experiment; a) Diagram of the multi-factorial experimental design. Black dots represent the location of *C. fornicata* within the tub, solid brown colour represents the sediment used for burial. For experiment 1, each trial consisted of one control individual (unburied) and three treatment individuals buried to 2, 6 and 12cm. Each trial of four individuals was repeated for three burial durations of

2, 7 and 20 days. Each trial was replicated 5 times (n=60, Control n= 15). Experiment 2 used the same protocol as above but stacks were used rather than individuals, and there were 4 replicates (n=48, Control n= 12). b) The layout of 30 tubs in tank 2 of experiment 1. Burial depths are shown in black text and burial duration in days is shown in yellow text. C = Control

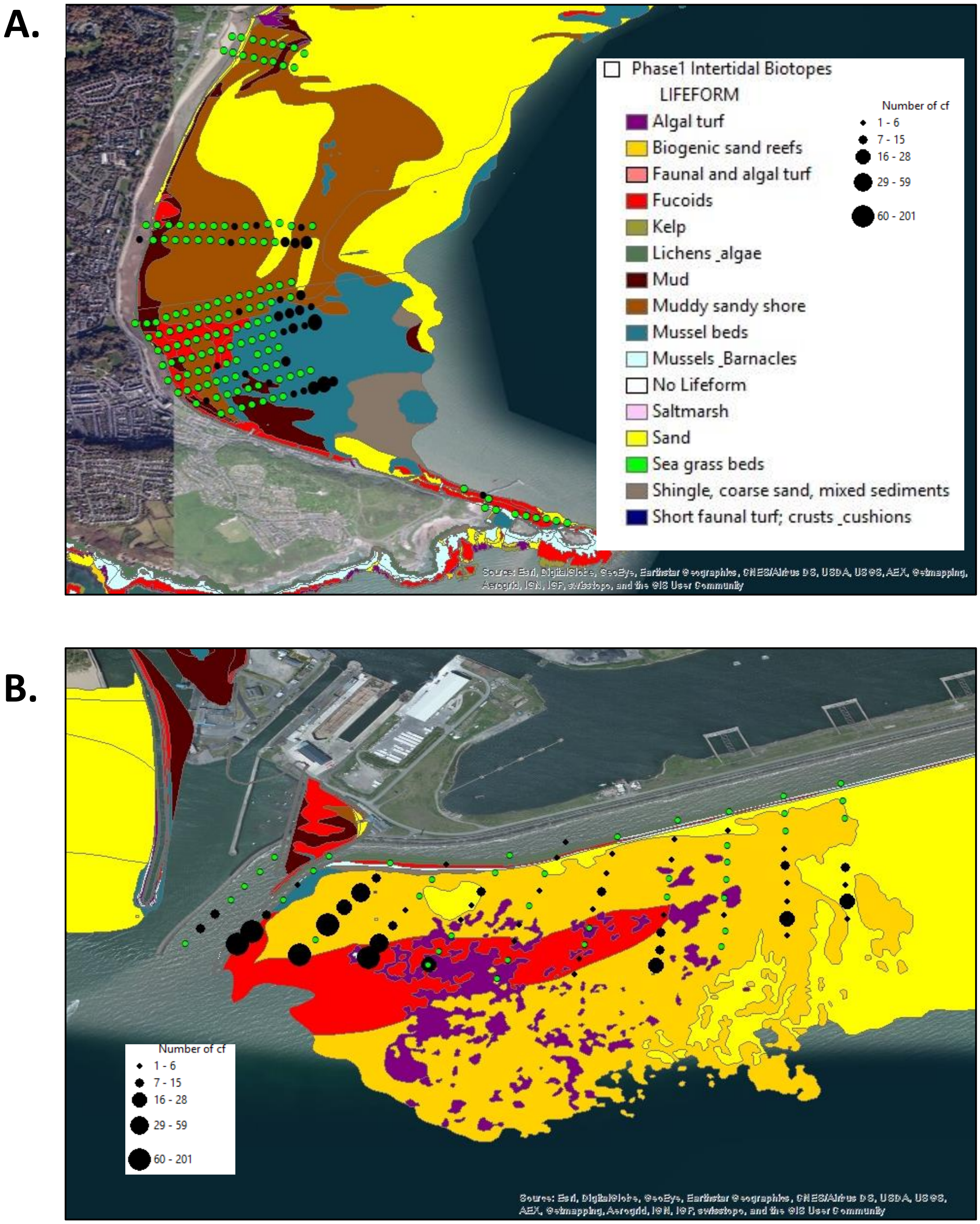
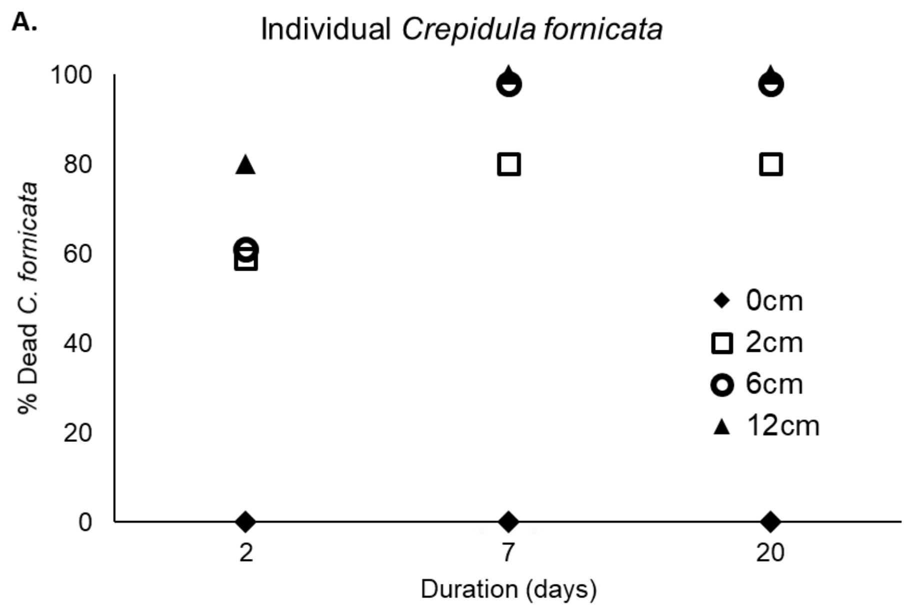


Figure 5. The presence and absence of *Crepidula fornicata* in intertidal areas of Swansea Bay. Green dots show stations surveyed where no *C. fornicata* was recorded. Black dots show stations where *C. fornicata* was found to be present; the size of dot indicates abundance (legend 'Number of cf'). The Phase I map shows the biotopes associated with each area (Countryside Council Wales 2003/2004).



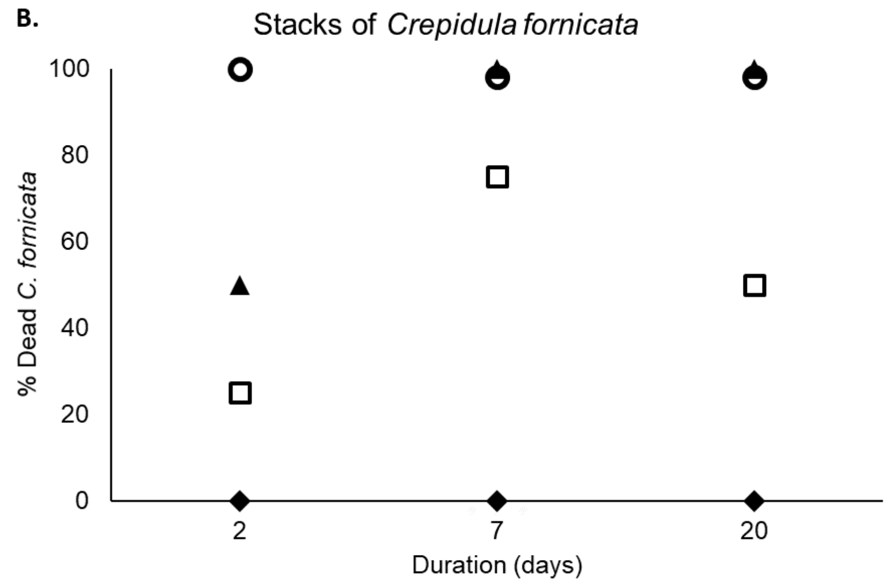


Figure 6. Laboratory trials assessing the survival of *Crepidula fornicata* under different sediment burial scenarios. Exposure to combinations of different sediment thickness (0-12 cm) and duration (2-20 days) were measured (A. individuals, 15 individuals tested per treatment; B. stacks, 12 stacks tested per treatment).

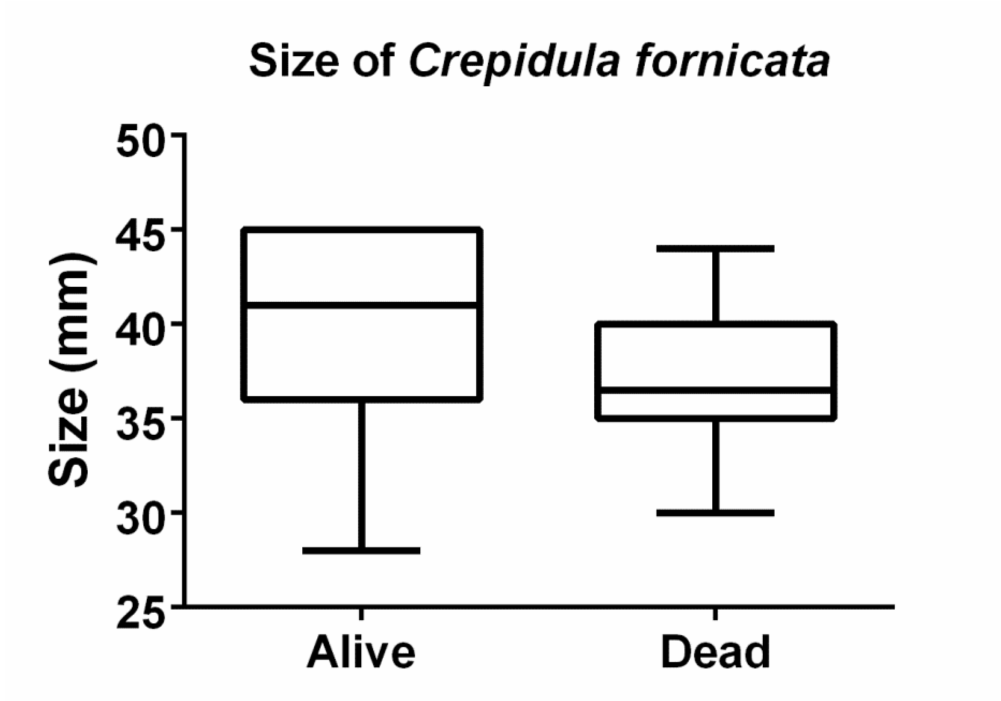


Figure 7. The size of *Crepidula fornicata* individuals in experiment 1 (alive n=7, dead n=38).